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Effects of Processing on Proximate Composition of *Hibiscus rosa-sinensis* Leaf

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Authors' contributions

This work was carried out equally, in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Leaves of *Hibiscus rosa-sinensis* are processed using different methods depending on the intended application. Using three different processing methods, we investigated the effects of processing on the proximate constitution of the leaf. Result demonstrated that the fresh raw leaf had moisture content of 82.30 ± 0.42%, which were significantly ($p<0.05$) reduced by drying but not extraction and blanching. The protein content of the raw leaf was low (1.80 ± 0.10%). Extraction and blanching reduced the protein content, whereas drying increased the protein content significantly ($p < 0.05$) for raw dried leaf powder and blanched leaf products. The raw leaf contained vitamins A, $B_{2,}$ C and E, which were significantly reduced by extraction and blanching, but were concentrated by drying. Anti-nutrient contents of the raw leaf were low and were reduced to negligible levels by the processing techniques employed. Comparing the nutrient and chemical constituents with recommended dietary allowance (RDA) values; we found that the leaves contain an appreciable

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amount of nutrients, minerals, vitamins, proteins and phytochemicals and low degree of toxicants. These findings suggested that the treatment method employed in processing this leaf affected the proximate composition, and this should be considered in utilization of this leaf (and other leaves) product in various food and pharmaceutical formulations.

Keywords: Blanching; extraction; processing methods; nutraceutical potential; proximate analysis.

1. INTRODUCTION

Interest in the relationship between diet and health has increased the demand for more information on proximate composition of different food sources. Credible scientific investigations have associated various potential health benefits with different food components. Similarly, advances in biotechnology, increasing health care costs, changes in food laws, product claims, aging population and rising interests in attaining wellness through diet, have fueled consumers interest in nutraceuticals. More foods are being fortified with nutrients and other physiologically active components for different nutritive and pharmacological roles including digestive, cardiovascular and respiratory health, boost in cellular antioxidant defenses, improvement in gastrointestinal health and systematic immunity among others [1-3].

Hibiscus rosa-sinensis is an ornamental plant widely grown throughout the tropics and sub-tropics. Numerous varieties, cultivars and hybrids are available. It is a traditionally applied medicinal herb, with high anti-oxidant and vitamins contents, and documented potencies in reducing cholesterol levels, decreasing chances of developing pyrexia, liver and cardiovascular disease, as well as anti-cancer and antihypertensive properties [4-6]. It is also known to block adipogenesis [7]. Tea made from hibiscus flowers and, occasionally, leaves is a very common beverage in the tropics. The cool, astringent, acidic flavour is widely recognized and has made it staple of Zinger type of teas in the United States. Documented evidences suggest that extracts of hibiscus have shown therapeutic properties *in vitro* and *in vivo*, including reduction of skin cancer promoted by ultraviolet light, inhibition of herpes simplex virus, wound healing, hypo-cholesterolemia and anti-bacterial activities [8-10].

Previously, we reported that various leaf products of *H. rosa-sinensis* possess protective potentials against metabolic syndrome related disease conditions [11]. In this study, we investigated how the various methods (drying, blanching and extraction) employed in processing *Hibiscus rosa-sinensis* leaves affected the phytochemical constitution vis-a-viz its nutritive and anti-nutritive properties.

2. MATERIALS AND METHODS

Preparation of the leaves is as reported previously [11] and summarized below. Leaves of *Hibiscus rosa-sinensis* were procured, based on ethno-pharmacological information, from the Department of Animal Science, and identified in the Department of Plant Science and Biotechnology, The University of Nigeria, Nsukka; by a taxonomist, Cyprian Okafor. A portion of the leaves was deposited in the Departmental herbarium for reference. Fresh leaves were harvested, washed with distilled water and drain-dried for few minutes. The leaves were divided into nine (9) portions of 100 g each. The 1st portion was kept for analysis as the raw leaf (RL) control. The 2nd portion was blended with water, filtered and the filtrate pasteurized at 70℃ for 30 minutes hereafter referred to as raw leaf extract (RLE). The 3rd, 4th and 5th portions were blanched in hot water at 100℃ for 2, 4 and 6 minutes, respectively, after which they were blended with water, filtered and the filtrate pasteurized at 70℃ for 30 minutes and respectively, called 2 minutes blanched leaf extract (B_2LE), 4 minutes blanched leaf extract (B_4LE) and 6 minutes blanched leaf extract (B_6LE). The 6th portion was dried at 50℃, milled into powder and called raw dried leaf powder (RDLP). The 7th, 8th and 9th portions were blanched in hot water at 100℃ for 2, 4 and 6 minutes respectively, dried at 50℃, milled into powder and called 2 minutes blanched dried leaf powder (B_2DLP), 4 minutes blanched dried leaf powder (B_4DLP) and 6 minutes blanched dried leaf powder (B_6DLP), respectively.

All chemicals used were of analytical grade and purchased from Sigma Aldrich (Seelze, Germany). Crude mineral content were determined using the methods reported previously [12]. Crude Protein was determined by the Kjeldahl method using 0.2 g of the weighted sample. Crude ash content was determined

charring triplicate samples (1 gram) on a hot plate under the fume cupboard. The charred material was incinerated in the muffle furnace at 550℃ until a whitish grey ash was obtained, cooled in a desiccator and weighed. Soxhlet extraction method was employed for determination of crude fat as follows: Five (5) grams of the sample was weighed into extraction thimble and covered with light layer of cotton wool. A round bottom flask was weighed and 150 ml light petroleum ether (boiling point of 60-80℃) was poured into the flask and refluxed for 6 hours, drained and dried at 100℃ for two hours in a hot air oven, then cooled and weighed. To determine the amount of crude fiber present, three (3) grams of the sample were weighed into a 50 ml beaker and the fat component was extracted with petroleum ether, air-dried and transferred to a 600 ml dried beaker. Two hundred (200) ml of 1.25% Sulphuric acid and few drops of anti-foaming agent were added, and then digestion apparatus with pre-adjusted hot plate before boiling for 30 min with periodic rotation. The mixture was allowed to stand for one minute and was filtered immediately in a Buchner funnel. Without breaking suction, the insoluble matter was washed with boiling water until it was free of the acid. The residue was washed back into the original flask using 200 ml of 1.25% sodium hydroxide solution reboiled for 30 min and again stood for one minute and then filtered under suction. The residue was washed with boiling water, followed by 1.0% hydrochloric acid and finally with boiling water until it was free of acid. It was washed twice with alcohol and then with ether for about three times. The residue was transferred into ashing dish and dried at 100℃ to a constant weight. Incineration to ash was done at 600℃ for 30 minutes and it was cooled in a desiccator and weighed. The difference in weight between oven dry weight and the weight after incineration was taken as the fiber content of the sample. The percentage carbohydrate content of the samples was determined as follows:

% Carbohydrate = 100 – (% moisture + % ash + % protein + % fat + % fibre)

The AOAC method [12] using the colorimeter was adopted for Determination of Vitamin A. Vitamin E was determined applying previous protocols [13], while to determine Vitamin C; sample (20 g) was extracted (blended) with 200 ml of 6% trichloroacetic acid solution and filtered, as described previously [12] with little modifications. Small quantity of animal charcoal was added to the filtrate to decolorize it a little. One milliliter of the filtrate was pipetted into a test tube. Ascorbic acid standard (1 ml) was also pipetted into another test tube and 1 ml of 6% trichloroacetic acid pipetted into a third test-tube as a blank. Dinitrophenyl hydrazine-theourea-copper sulphate (1 ml) reagent was added to all the test tubes, capped, mixed and incubated in 37˚C water bath for 3 hours. The tubes were then chilled for 30 min in an ice bath with slow agitation. Then 12 M H_2SO_4 (2 ml) was added. Absorbance (at 520 nm) was read using a Beckmann spectrophotometer. Ascorbic acid (Vitamin C) stock was used as standard. Riboflavin was determined using the method described in the AOAC protocol [12]. Mineral composition was determined by Atomic Absorption Spectrophotometry [14]. The alkaline titration method, the titration method, solvents extraction, and the Folin-Denis Colorimetric method were employed for determination of the concentrations of Hydrogen Cyanide (HCN), oxalates, Saponin and Tannin respectively as described in previous protocols [12,14].

2.1 Statistical Analysis

All results were expressed as Mean ± Standard deviation (S.D.) of independent triplicate experiments. Data were statistically analyzed using one way Analysis of Variance (ANOVA) in a completely randomized design (CRD). The level of Significance was set at $P < 0.05$ and difference between means were determined with least significance difference (LSD) according to Steele and Torrie [15].

3. RESULTS

The results of the proximate composition of the samples are shown in Table 1. The moisture content ranged from 6.67% (w/w) to 84.53% (w/v) with raw leaf extract (RLE) having the highest moisture content (84.53%). When the raw leaf components were extracted with water, the raw RLE extract (RLE) contained significantly ($p < 0.05$) higher amount of moisture than the raw leaf (RL). Blanching of the leaf before extraction with water also led to significantly ($p < 0.05$) higher moisture. The higher moisture content of the extracts could be attributed to additional water used during extraction because the leaf is naturally highly slimy or rather contain mucilaginous substances (a characteristics that makes extraction difficult). The raw leaf extract (RLE) contained significantly ($p < 0.05$) higher amount of moisture than those blanched before

extraction. Although the raw leaf extract (RLE) contained significantly ($p < 0.05$) higher amount of moisture, no significant difference ($p > 0.05$) in moisture content could be found between B_4LE and B_6LE. The lower moisture content of the samples blanched before extraction compared to the raw leaf extract (RLE) could be attributed to losses arising from heat of blanching [16,17]. The moisture contents of the dried powdered products were lower than the raw leaf (RL) with the raw dried leaf powder and six minutes blanched leaf extract (B_6DLP) having the highest and lowest moisture content respectively. These results revealed that the raw dried leaf powder (RDLP) was significantly higher ($p < 0.05$) than the blanched samples. Fibre contents of the products ranged from not detectable (ND) to 3.152%. Raw dried leaf powder (RDLP) had the highest fiber (3.152%) content compared with the subsequent products.

Lipid content of the raw leaf was 0.51 ± 0.007%, and this was within the range for leafy vegetables reported previously [16,18,19] but was below the range for some leafy vegetables reported by others [17,20-22]. The leaf extracts were low in lipid content and significantly ($p < 0.05$) lower than the fat content of the raw leaf (RL). Among the leaf extracts, the extract from the raw leaf (RLE) was higher in fat content than the other extracts namely B_2LE, B_4LE and B_6LE. The differences could be attributed to the effect of blanching [16,17,23] as it appeared that some of the lipid was lost during blanching; hence, the longer the blanching time, the lower the lipid content. The lipid contents of the dried products were also low but significantly ($p < 0.05$) higher than the raw leaf or the extracts. The raw dried leaf powder had the highest lipid content. It is expected that drying would increase the lipid contents per unit weight due to concentration resulting from moisture loss while losses due to blanching (a pre-drying treatment) seem to have affected the blanched dried samples.

There were some significant variations in the level of protein in the leaf products as their protein content ranged from 0.94 to 2.60%, with the raw leaf (RL) having crude protein content of 1.80 ± 0.014%. These protein content values were far below the range obtained from other leafy vegetables as reported by other research groups [17, 22, 24] but were within the range obtained from Kenaf and Corchorus leaves [19], *H. sabdariffa* [18] and *Telfaria accidentalis* [16]. Extraction significantly ($p < 0.05$) reduced the protein content of the leaf products. This explains why the extracted products had lower protein content than the raw leaf. Among the leaf extracts, the protein level of RLE was significantly higher ($p < 0.05$) than the B_2LE, B_4LE and B_6LE. This suggests that blanching also contributed to loss of protein in the extracted products. Ash content of the processed products ranged from 2.48 to 11.81% with the raw leaf (RL) having a value of 2.48 ± 0.035%. The ash contents of the raw leaf (RL) was higher than the ash content of some leafy vegetables reported by Mepba et al. [16], Gupta and Prakash [25], Shokunbi et al. [21] but were within the range of some other leafy vegetables reported by Awogbemi and Ogunleye [18], Nkafamiya et al. [17] and were lower than those reported by Akubugwo et al. [26], Lewu et al. [27], and Mwanri et al. [22]. Extraction caused a decrease in the ash content of the leaf. This is why the raw leaf (RL) ash content was higher than those of the raw leaves extracted products. This suggests that losses of minerals occurred during the extraction process either due to difficulties of extracting all the minerals from the leaf matrix or due to other problems such as adherence to the materials removed during filtration. Among the extracts, the raw leaf extract (RLE) contained significantly ($p < 0.05$) more minerals, than the blanched leaf extracts. Ash content was also found to be decreasing with increasing blanching time. This is why the two minutes blanched leaf extract (B_2LE) had significantly ($p < 0.05$) higher ash content than the six minutes blanched leaf extract (B_6LE). Drying appeared to concentrate the ash content per unit weight. The ash content of the raw dried leaf powder (RDLP) sample was found to be 11.18 ± 0.042%. This value was found to be significantly ($p < 0.05$) higher than the ash content of the raw leaf (2.48 ± 0.035%). Among the dried leaf products, the raw dried leaf powder (RDLP) was found to contain significantly ($p < 0.05$) the highest ash content as blanching before drying reduced the ash content. Consequently, longer blanching time before drying, caused lower ash content. This is why the six minutes blanched dried leaf product (B_6DLP) had the lowest ash content compared to the other products dried after blanching.

The carbohydrate contents of the extracts were significantly ($p < 0.05$) higher than that of the raw leaf. On extraction, the carbohydrate content increased from 10.58 ± 0.41% in the raw leaf (RL) to 13.66 ± 0.098% in the raw leaf extract (RLE). It is probable that some of the fibre components were disintegrated during the process of extraction, thereby contributing to the carbohydrate content by being part of the filtrate.

Table 1. Proximate composition of processed samples from *Hibiscus rosa-sinensis* leaves (%)

Parameters / Samples	Moisture (%)	Ash (%)	Crude protein (%)	Crude fibre (%)	Fat (%)	Carbohydrate (%) (by difference)
RL	82.30^{e}± 0.42	2.48^{e}±0.035	1.80^{e}±0.014	1.52^{a}±0.042	0.510^{e}±0.007	10.58^{a} ± 0.41
RLE	84.53^{h}± 0.11	0.36^{d}±0.014	1.20^{d}±0.007	ND	0.245^{d}±0.007	13.66^{b} ± 0.098
B_2LE	83.93^{g}± 0.69	0.205^{c}±0.007	0.96^{b} ± 0.01	ND	0.220^{c}±0.000	14.68^{c} ± 0.714
B_4LE	83.42^{f}±0.003	0.175^{b}±0.007	0.95^{c}±0.000	ND	0.210^{b}±0.014	15.24^{d} ± 0.014
B_6LE	83.32^{f} ± 0.10	0.130^{a}±0.042	0.94^{a}±0.000	ND	0.190^{a}±0.014	15.41^{e} ±0.04
RDLP	7.70^{d}± 0.014	11.18^{i}±0.042	2.60^{i}± 0.014	3.15^{e}±0.021	1.58^{i} ± 0.028	73.81^{f} ± 0.127
B_2DLP	7.51^{c}± 0.056	10.80^{h}±0.014	2.35^{h}±0.002	3.04^{d}± 0.09	0.77^{h} ± 0.028	75.52^{g} ± 0.141
B_4DLP	7.24^{d}± 0.014	10.18^{a} ± 0.02	1.96^{g}±0.014	2.88^{c} ± 0.02	0.670^{g}±0.040	77.07^{h} ± 0.07
B_6DLP	6.67^{a}± 0.19	9.59^{f} ± 0.02	1.90^{f}±0.014	2.81^{b}±0.014	0.625^{f} ± 0.02	78.41^{i} ± 0.155

Values are means ± standard deviation of three determinations. Values on the same column with different superscripts are significantly different ($p < 0.05$), while those with same superscripts are not ($p>0.05$). RL = Raw leaf (overall control), RLE = raw leaf extract, B_2LE = 2 minutes blanched leaf extract, B_4LE = 4 minutes blanched leaf extract, B_6LE = 6 minutes blanched leaf extract, RDLP = raw dried leaf powder (control), B_2DLP = 2 minutes blanched dried leaf powder, B_4DLP = 4 minutes blanched dried leaf powder, B_6DLP = 6 minutes blanched dried leaf powder. ND = Not Detected

Table 2. Vitamin composition of processed samples from *Hibiscus rosa-sinensis* leaves (mg/100 g)

Parameters / Samples	Vitamins			
	Vitamin A	Vitamin B_2	Vitamin C	Vitamin E
RL	2.700^{a} ± 0.014	1.985^{a} ± 0.007	42.105^{a} ± 0.007	12.800^{a} ± 0.000
RLE	1.035^{d} ± 0.021	1.090^{e} ± 0.014	38.895^{b} ± 0.021	3.610^{f} ± 0.014
B_2LE	0.970^{e} ± 0.014	0.940^{f} ± 0.014	36.950^{c} ± 0.014	3.105^{g} ± 0.007
B_4LE	0.940^{f} ± 0.014	0.885^{g} ± 0.021	36.110^{d} ± 0.014	2.990^{h} ± 0.014
B_6LE	0.925^{f} ± 0.007	0.840^{h} ± 0.014	33.940^{e} ± 0.127	2.965^{h} ± 0.021
RDLP	2.560^{b} ± 0.014	1.515^{b} ± 0.021	20.430^{f} ± 0.028	10.110^{b} ± 0.041
B_2DLP	2.500^{c} ± 0.014	1.220^{c} ± 0.028	17.080^{g} ± 0.325	10.005^{c} ± 0.007
B_4DLP	2.485^{c} ± 0.000	1.185^{cd} ± 0.007	16.130^{h} ± 0.042	9.940^{d} ±0.014
B_6DLP	2.470^{c} ± 0.000	1.150^{d} ± 0.014	14.135^{i} ± 0.247	8.765^{e} ± 0.007

Values are means ± standard deviation of three determinations. Values on the same column with different superscripts are significantly different ($p < 0.05$), while those with same superscripts are not ($p>0.05$). RL = Raw leaf (overall control), RLE = raw leaf extract, B_2LE = 2 minutes blanched leaf extract, B_4LE = 4 minutes blanched leaf extract, B_6LE = 6 minutes blanched leaf extract, RDLP = raw dried leaf powder (control), B_2DLP = 2 minutes blanched dried leaf powder, B_4DLP = 4 minutes blanched dried leaf powder, B_6DLP = 6 minutes blanched dried leaf powder

Results (Table 2) demonstrated that raw leaf (RL) had the highest vitamin A content than the rest of the processed samples. The fresh raw leaf had a vitamin A content of 2.7 ± 0.014 mg/100 g sample. When this value was compared to other leafy vegetables, it was obvious that the Vitamin A content of the processed raw leaf was very low. Extraction resulted to reduction of the vitamin A of samples. This is exemplified by the raw leaf extract (RLE), which was significantly ($p < 0.05$) lower (1.035 ± 0.021 mg/100 g) than the raw leaf. Blanching caused further losses of vitamin A in the leaf. The two minutes blanched leaf extract (B_2LE) had lower vitamin A content (0.97 ± 0.014 mg /100 g sample) than the raw leaf (RL) or raw leaf extract (RLE). Longer blanching time was also associated with higher vitamin A losses. Hence, the two minutes blanched leaf extract (B_2LE) had significantly ($p < 0.05$) high vitamin A content than the six minutes blanched leaf extract (B_6LE). The greater reduction of vitamin A in the blanched samples is attributed to effects of heat and leaching accompanying blanching. Drying also resulted to lower content of vitamin A in the samples showing that losses occurred during drying. Thus, raw dried leaf powder (RDLP) was significantly ($p < 0.05$) lower than the raw leaf (RL). Blanching before drying also contributed to losses of vitamin A in the dried products; and this effect increased in time dependent manner. *Riboflavin* levels present in the processed products ranged from 0.84 to 1.985 mg/100 g. It can be deduced that the Vitamin B_2 content of the products was within the range obtained from other leafy vegetable; such as *B. coastum, C. tora, F. trihopoda, M. oleifera* respectively [17] but was slightly lower than that of *Amarathus hybrius* leaves (4.24 mg/100 g) reported by Akubugwo et al*.* [26]. The raw leaf (RL) had significantly ($p < 0.05$) the highest content of riboflavin (1.985 ± 0.007 mg/100 g) compared with other samples (Table 2). All the samples had vitamin B_2 concentration significantly ($p < 0.05$) different from each other except that the sample blanched for four minutes followed by drying (B_4DLP) was not found to be significantly different ($p > 0.05$) from that blanched for two minutes followed by drying (B_2DLP) and that blanched for six minutes followed by drying (B_6DLP) but B_6DLP was found to be significantly ($p < 0.05$) different from B_2DLP. *Ascorbic acid* composition in the processed products ranged from 14.135 to 42.11 mg/100 g sample. The raw leaf (RL) had the highest vitamin C content level (42.105 ± 0.007 mg/100 g sample) compared to other processed products. Each processed product significantly ($p < 0.05$) differed from the other in vitamin C content. Among the dried leaf samples, the raw dried leaf product (RDLP) had the highest quantity of vitamin C. Others were lower due to the effects of blanching and the higher the blanching time, the lower the quantity of vitamin C retained. Hence, B_2DLP had significantly ($p < 0.05$) higher vitamin C content than B_6DLP. Generally, vitamin C contents of the products ranged thus: Raw leaf > Extracted samples >Dried samples. The longer the blanching treatment, the lower the vitamin C content as observed previously on the blanched dried samples. For Vitamin E values ranged from 2.97 to 12.80 mg/100 g sample. The raw leaf (RL) had a concentration of 12.8 ± 0.00 mg/100 g and this compares favorably with the results from work reported by Akubugwo et al*.* [26], Nkafamiya et al*.* [17] and Shokunbi et al*.* [21]. The results showed that raw leaf (RL) had the highest Vitamin E content (12.80 mg/100 g) compared to other samples. Among the leaf extracts, the raw leaf extract (RLE) vitamin E content was significantly higher than the blanched samples. Thus, we inferred that the longer the blanching time, the lower the vitamin E content. Similarly, the vitamin E contents of the extracts were lower than those of the dried leaves. As observed for the extracts, the longer the blanching time of the samples before drying, the lower the vitamin E content.

The calcium content (Table 3) of the raw leaf (RL) was within the range for some indigenous soup vegetables as previously reported for other parts of Nigeria [16]. The raw dried leaf powder (RDLP) had significantly ($p < 0.05$) higher calcium content compared to other samples. The blanched dried leaf samples possessed lower calcium content than the raw leaf. Among the dried samples, the raw dried leaf powder (RDLP), contained more calcium than the samples blanched before drying. Thus, longer blanching time was associated with lower calcium content of the samples probably due to leaching of the samples during blanching. This may account for why sample blanched for 6 minutes before drying (B_6DLP) had the lowest quantity of calcium among the dried samples. The extracted samples also contained lower quantity of calcium compared to the raw leaf (RL). Among the extracted samples, there were no significant ($p > 0.05$) differences in the quantities of calcium present. The lower amount of calcium in the extracted samples could be due to a number of factors including leaching during blanching, dilution effect by extracting water and

difficulty of extracting all calcium from the leaf matrix. Similarly, the potassium content of the raw leaf (RL) was found to be 4.84 ± 0.014 mg/100 g sample (Table 3). These results corroborate previous reports of Mepba et al. [16]. The potassium content of the raw leaf (RL) was significantly ($p < 0.05$) higher than those of the processed samples except that of raw dried leaf powder (RDLP). Among the dried samples, the raw dried leaf powder (RDLP) was significantly ($p < 0.05$) higher than the blanched dried leaf samples (B_2DLP, B_4DLP and B_6DLP). No significant ($p > 0.05$) difference was observed between raw leaf (RL) and 2 minutes blanched dried leaf powder (B_2DLP). The lower value of the blanched dried leaf powder (B_4DLP and B_6DLP) compared to raw leaf (RL) and raw dried leaf powder (RDLP) has been attributed to losses during blanching. The extracted samples were lower in potassium compared to the raw leaf. Leaching during blanching as well as difficulties of extraction could be responsible for the lower potassium content of the extracted samples compared with the raw leaf. Among the extracted samples, the raw leaf extracts (RLE) had the higher value than that blanched for 6 minutes followed by extraction (B_6LE) but not significantly ($p > 0.05$) different from those blanched for 2 and 4 minutes (B_2LE and B_4LE) before extraction.

The iron content of the processed products (Table 3) revealed that their values ranged from 7.61 to 9.595 mg/100 g with RDLP having the highest level of iron content of 9.59 mg/100g samples. This was significantly higher ($p < 0.05$) than the iron content of other processed products. Among the dried samples, the raw dried leaf product (RDLP) was significantly ($p < 0.05$) different from some samples. The raw leaf (RL) was not significantly ($p > 0.05$) different from that blanched for 2 minutes followed by drying (B_2DLP) and 6 minutes blanched (B_6DLP) before drying. The dried samples generally contained more iron than the extracts. Among the leaf extracts, the raw leaf extract (RLE) was not significantly ($p > 0.05$) higher in iron content compared with the samples blanched for 2 minutes before extraction (B_2LE). Both samples were significantly higher than samples blanched for 4 and 6 minutes before extraction (B_4LE and B_6LE, respectively).

The result of oxalates composition (Table 4), of the processed products showed that the raw leaf (RL) had the highest value of 0.92 mg/100 g sample than the rest of the products as their values ranged from 0.135 to 0.895 mg/100 g. The oxalate content of the raw leaf extract (RLE) was significantly higher ($p < 0.05$) than that of B_2LE (0.17 mg/100 g), B_4LE (0.155 ± 0.007 mg/100 g) and B_6LE (0.135 ± 0.007 mg/100 g). The results show that oxalate content reduced with increase in time of blanching, suggesting that blanching process adversely affected the oxalate content of the leafy vegetables. Effects of blanching, dilution due to extracting with water and difficulty of extracting all components of the leaf may account for the lower oxalate contents of the extracts compared to the raw leaf. The oxalate levels in the dried powdered products ranged from 0.595 to 0.895 mg/100 g. Among the dried products, the oxalate level of the raw dried leaf products (RDLP) was found to be significantly ($p < 0.05$) higher (0.985 ± 0.007 mg/100 g) than those that were blanched which included B_2DLP (0.66 mg/100 g), B_4DLP (0.625 ± 0.007 mg/100 g) and B_6DLP (0.595 ± 0.007 mg/100 g). It is evident that blanching contributed to reduction of oxalate content of the dried products, since the longer the blanching time, the lower the oxalate contents. Comparatively, extraction led to greater reduction in the oxalate content of the products than drying. It is seen from Table 4 that the oxalate contents of the dried products were more than 3 times the oxalate contents of the extracts. This implies that extraction may be a more beneficial method in reducing oxalate in this leaf. From the results, it can be deduced that blanching had an adverse effect on the oxalate content [17] of the processed samples (blanched dried and blanched extracts).

Furthermore, we found that the tannin contents ranged from 0.045 mg/100 g to 0.20 mg/100 g. RL had the tannin content of 0.20 ± 0.1 mg/100 g sample. This is significantly ($p < 0.05$) higher than the tannin content of other samples. Among the extracted samples, the tannin level of RLE was significantly ($p < 0.05$) higher than that of B_4LE (0.70 mg/100 g) and B_6LE (0.045 ± 0.007 mg/100 g) but was similar to that of B_2LE (0.095 ± 0.007 mg/100 g), suggesting that the longer the blanching time, the lower the tannin content in the extracted samples. The tannin content of raw dried leaf was significantly ($p < 0.05$) higher than the rest of dried samples. These findings suggest that level of tannin in the processed samples was quite low compared to work done by Adeboye and Babajide [28] on Utazi, Ugu, Egwuro and Igbo. The saponin content was 0.19 mg/100 g, which was far lower than that of *Amaranthus hybridus* (1.68 mg/100 g). Processing lowered the saponin content further as seen

Table 3. Mineral composition of processed samples from *Hibiscus rosa-sinensis* leaves (mg/100 g)

Samples	Minerals		
	Potassium	Calcium	Iron
RL	$4.840^{b} \pm 0.014$	$8.220^{b} \pm 0.014$	$9.030^{b} \pm 0.028$
RLE	$3.630^{e} \pm 0.028$	$5.080^{e} \pm 0.000$	$7.720^{e} \pm 0.041$
B_2LE	$3.605^{e} \pm 0.007$	$5.005^{e} \pm 0.007$	$7.700^{e} \pm 0.000$
B_4LE	$3.550^{e} \pm 0.021$	$4.955^{e} \pm 0.007$	$7.645^{f} \pm 0.495$
B_6LE	$3.480^{f} \pm 0.028$	$4.910^{e} \pm 0.014$	$7.605^{f} \pm 0.007$
RDLP	$5.460^{a} \pm 0.014$	$9.300^{a} \pm 0.014$	$9.595^{a} \pm 0.021$
B_2DLP	$4.800^{b} \pm 0.014$	$6.500^{c} \pm 0.141$	$9.000^{b} \pm 0.000$
B_4DLP	$4.530^{c} \pm 0.028$	$6.255^{d} \pm 0.078$	$8.870^{c} \pm 0.028$
B_6DLP	$4.140^{d} \pm 0.085$	$6.100^{d} \pm 0.141$	$8.795^{d} \pm 0.021$

Values are means ± standard deviation of three determinations. Values on the same column with different superscripts are significantly different ($p < 0.05$), while those with same superscripts are not ($p>0.05$). RL = Raw leaf (overall control), RLE = raw leaf extract, B_2LE = 2 minutes blanched leaf extract, B_4LE = 4 minutes blanched leaf extract, B_6LE = 6 minutes blanched leaf extract, RDLP = raw dried leaf powder (control), B_2DLP = 2 minutes blanched dried leaf powder, B_4DLP = 4 minutes blanched dried leaf powder, B_6DLP = 6 minutes blanched dried leaf powder

Table 4. Anti-nutrients composition of processed samples from *hibiscus rosa-sinensis* leaves (%)

Parameter / Sample	Tannin	Saponin	Oxalate	Hydrogen cyanide
RL	$0.200^{a} \pm 0.01$	$0.190^{a} \pm 0.000$	$0.920^{a} \pm 0.028$	$0.650^{a} \pm 0.007$
RLE	$0.110^{c} \pm 0.000$	$0.135^{b} \pm 0.007$	$0.340^{f} \pm 0.000$	$0.350^{c} \pm 0.014$
B_2LE	$0.095^{c} \pm 0.007$	$0.115^{bc} \pm 0.007$	$0.170^{g} \pm 0.000$	$0.285^{e} \pm 0.007$
B_4LE	$0.070^{d} \pm 0.000$	$0.095^{c} \pm 0.007$	$0.155^{gh} \pm 0.007$	$0.250^{f} \pm 0.000$
B_6LE	$0.045^{e} \pm 0.007$	$0.080^{d} \pm 0.000$	$0.135^{h} \pm 0.007$	$0.235^{f} \pm 0.007$
RDLP	$0.155^{b} \pm 0.007$	$0.105^{c} \pm 0.007$	$0.895^{b} \pm 0.007$	$0.500^{b} \pm 0.000$
B_2DLP	$0.110^{c} \pm 0.000$	$0.090^{cd} \pm 0.007$	$0.660^{c} \pm 0.000$	$0.335^{c} \pm 0.007$
B_4DLP	$0.095^{c} \pm 0.007$	$0.075^{d} \pm 0.000$	0.625 ± 0.007	$0.305^{d} \pm 0.007$
B_6DLP	$0.075^{d} \pm 0.007$	$0.060^{e} \pm 0.014$	$0.595^{e} \pm 0.007$	$0.280^{e} \pm 0.000$

Values are means ± standard deviation of three determinations. Values on the same column with different superscripts are significantly different ($p < 0.05$), while those with same superscripts are not ($p>0.05$). RL = Raw leaf (overall control), RLE = raw leaf extract, B_2LE = 2 minutes blanched leaf extract, B_4LE = 4 minutes blanched leaf extract, B_6LE = 6 minutes blanched leaf extract, RDLP = raw dried leaf powder (control), B_2DLP = 2 minutes blanched dried leaf powder, B_4DLP = 4 minutes blanched dried leaf powder, B_6DLP = 6 minutes blanched dried leaf powder. ND = Not detected

in the dried leaf samples and extracted leaf samples. On drying the raw leaf (RDLP), the saponin reduced to 0.105 ± 0.007 mg /100 g samples. When the leaf was blanched before drying, the saponin content reduced even more as shown by B_2DLP (0.09 ± 0.007 mg/100 g), B_4DLP (0.078 ± 0.00 mg/100 g) and B_6DLP (0.06 ± 0.014 mg/100 g). This shows that the longer the blanching time, the greater the reduction in the saponin contents of the samples. Similarly, extraction processes reduced the saponin content of samples. This is evident in the saponin of the raw leaf extract (RLE = 0.135 ± 0.007 mg/100 g) compared to the raw leaf (RL). Blanching further enhanced the effect of extraction in reducing the saponin content of products. This is why the blanched samples had even lower saponin contents and the longer the blanching time the lower the saponin contents in the extracted products. For Hydrogen cyanide (HCN), the Raw leaf (RL) concentration was 0.65 ± 0.007 mg/100 g sample. This is slightly lower than the range (0.86 - 1.35 mg/100 g sample) reported by Oboh [20]). Processing of the leaves further reduced the cyanide content of the products. Drying reduced the cyanide content of the raw leaves to 0.5 ± 0.00 mg/100 g sample (RDLP). Blanching before drying caused a further reduction of cyanide in the dried powdered leaf products. Hence, samples blanched for 6 minutes followed by drying had cyanide concentration of 0.28 mg/100 g sample and this was found to be significantly ($p < 0.05$) lower than the concentration in the raw leaf, 0.650 mg/100 g and raw leaf dried powder, 0.500 mg/100 g. Longer blanching time was associated with greater reduction in the cyanide contents in the dried leaf products. This is evidenced by the

cyanide content of the samples blanched for two minutes before drying which was significantly ($p < 0.05$) higher than that blanched for 6 minutes before drying. Nkafamiya et al. [17] similarly observed that blanching reduces the cyanide content of some vegetables. Extraction also reduced the cyanide content of the leaf products. This is shown in the raw leaf extract with a concentration of 0.35 ± 0.014 mg/100 g sample, which was significantly ($p < 0.05$) lower than that of the raw leaf. Also, blanching contributed significantly ($p < 0.05$) to the lowering of cyanide content of the extracted products. Longer blanching periods were associated with greater reduction in the cyanide contents of the extracts. Thus, sample blanched for 2 minutes followed by extraction had a concentration of 0.235 ± 0.007 mg/100 g.

4. DISCUSSION

High moisture content in leafy vegetables, such as *H. rosa-sinensis* leaves, is indicative of freshness, easy perishability and vulnerability to microbial attack. In the tropics, wastage of vegetable crops is estimated to be around 50% due to high moisture content compared to 10 to 25% in developed countries where vegetable are processed to minimize losses [27]. Our results demonstrated that the moisture content of the fresh/raw leaf (RL) was within the range expected for fresh vegetables as reported previously [16,18,20,21,24,27], though differed from results obtained by other investigators [17]. The lower moisture content of the dried leaf powdered products could be attributed to the effects of dehydration or moisture loss. This low moisture content of the dried powdered samples is an advantage over the rest of the processed products as the decreased moisture level would lead to concentration of soluble solids that make up the nutrients [16], as well as extend the shelf life of the vegetable. Fibre content of the processed products were far lower than the 0.5 to 10.9%, 13.80% (DW) and 22.84% (DW) reported by Awogbemi and Ogunleye [18], Akubugwo et al. [26], Lewu et al. [27] and Hussain et al. [24] but were within the range reported by Shokunbi et al. [21]. The fibre content of the raw leaf was on the same range with that of *H. sabdariffa* (Sorrel Isapa) another specie of Hibiscus plant [18]. The difference between the fibre content of the raw leaf of *H. rosa-sinensis* and that of other leafy vegetables could be due to variations in the environmental factors and agronomic practices at the various locations where the analyzed leaves were harvested [21]. When the raw leaf was dried, the fibre content significantly ($p < 0.05$) increased, probably due to moisture removal, which concentrated other components per unit weight including the fibre contents. Hence; RDLP, B_2DLP, B_4DLP and B_6DLP contained significantly ($p < 0.05$) higher quantities of fibre than RL.

Among the dried powdered samples, the RDLP was higher in fibre than the blanched counterparts (B_2DLP, B_4DLP and B_6DLP) but no significant ($p > 0.05$) difference existed between samples blanched for 4 and 6 minutes before drying. The level of fibre in the dried leaf powdered samples was lower compared to what was obtained from the leaves of the same plant, *H. rosa-sinensis* in India on dry weight basis [29]. Although dehydration does not decrease (rather it increases) the fibre content of leafy vegetables [16,18,19], blanching decreases fibre content of vegetables [17]. The slight decrease in the fibre content of the blanched dried samples compared to unblanched could be attributed to hydrolysis caused by heat of blanching. No fibre could be detected from the extracts either due to filtration or dilution with water or both. The level of fibre in these extracted products was generally low. Fibres of leafy vegetables have been reported to possess therapeutic potentials against colon cancer, gastrointestinal disorders [21]; obesity, diabetes, cancer [27]; promote soft stools with increased frequency and regularity of elimination [30]; lowers serum cholesterol level, reduces risk of coronary heart diseases and hypertension [26]. Moreover, the processed leaf products were poor sources of lipids. This agrees with the findings that leafy vegetables are low lipid containing food and this would be advantageous to obese patients [26]. Longer blanching time correlated with lower protein content, while drying caused an increase in the protein content of the leaf. The raw dried leaf powder (RDLP) had significantly ($p < 0.05$) higher protein compared to the raw leaf, probably due to concentration effect resulting from moisture loss. Although all dried leaf products had significantly ($p < 0.05$) higher protein content compared to the raw leaf, blanching before drying appeared to cause lower protein content. Comparatively, each dried product was significantly ($p < 0.05$) higher in protein content compared to the equivalent extracted counterpart. Since the crude proteins content of the products were low, this leaf cannot be regarded as a good source of protein. Ash level, which is usually a measure of the nutritionally important mineral content, in the

dried leaf samples was within the range previously obtained from the leaves of the same plant on dry weight basis [29]. Carbohydrate content of the raw leaves was within the range of that of *Amaranthus hybridus* and *Laurea taraxifolia* [18] but was higher than the range of carbohydrate for other vegetables in literature. Therefore, since the carbohydrate content of *H. rosa-sinensis* leaf is high, it can be used as a good source of energy. Increase in blanching time before extraction also led to increase in carbohydrate content of the extract. This suggests that much of the fibre components were hydrolyzed by heat of blanching, in addition to disintegration during extraction, all leading to increase in the carbohydrate content of the extracts. The results also show that the longer the blanching time, the higher the carbohydrate content.

The high carbohydrate content of the dry powdered products could be attributed to the effect of drying which concentrated the carbohydrate into a higher mass as moisture was removed as reported previously [18,16]. Blanching contributed to remarkable increase in the carbohydrate content of the blanched dried as already explained for the extracted products. The Recommended dietary allowance (RDA), values for children, adults; and pregnant and lactating mothers are 130 g, 130 g, 175 g and 210 g respectively. This implies that 40, 40, 30 and 25% of their respective daily requirement can be met when 100 g of dried *Hibiscus rosa-sinensis* leaves are consumed [26]. The carbohydrate range for the dry matter of the processed product was within the range for sweet potatoes leaves (82.8%) and C*orchorus tridens* (75.0% DW) reported by Akubugwo et al*.* [26]. The processed products especially the dry powdered products were starchy vegetables and so could be promising source of energy. The vitamin A content of these processed products were slightly lower than that of *Amarathus hybridus* (3.29 mg/100 g) reported by Akubugwo et al*.* [26], *C. tridens, A. spinosus* (2.71 and 2.75 mg/100 g, respectively) reported by Nkafamiya et al*.* [17]. Also, the decrease in the Vitamin A level of the blanched samples could be attributed to effect of blanching [17]. Comparatively, greater losses of vitamin A occurred in the extraction process than drying process. Thus, the dried samples had significantly ($p < 0.05$) higher vitamin A content than the corresponding extracted products. The lower vitamin B_2 content of the dried leaf samples compared to the raw leaf could be attributed to loss due to blanching and drying. Gupta and Prakash [25] reported previously that vitamins, especially the water-soluble vitamins, are more prone to destruction on drying. The vitamin B_2 contents of the extracted products differed significantly ($p<0.05$) from each other. Among the extracted products, the raw leaf extract (RLE) had significantly ($p < 0.05$) the highest vitamin B_2 content. However, all the extracts had significantly ($p < 0.05$) lower vitamin B_2 content compared to the raw leaf. It is concluded that both heat of blanching, difficulty of extracting all vitamins from leaf matrix and dilution from the extracting water could account for the lower vitamin B_2 content of the extracts compared to raw leaf. Though blanching is known to inactivate enzymes in vegetable, causing vitamin losses by thermal degradation, diffusion and leaching in the process [31]. However, the vitamin C content of the raw leaf (RL) of this vegetable was slightly lower than that given for other vegetable, which ranged from 48 to 220 mg/100 g [16,32]. Loss of Vitamin C in these processed products appears to be a function of the processing method employed in its preparation as noted by Babalola et al*.* [32]. Blanching appeared to greatly reduce the vitamin E content of the products. According to Morris et al. [33], blanching increases the rate of oxidation of vitamin E. Vitamin E is a known antioxidant and a strong performer of other cellular functions [34]. We hypothesize, thus, that *H. rosa-sinesis* leaf is a potential source of dietary vitamins for nutrition. Results on Calcium compositions agree with those of Mepba et al*.* [16]. Calcium is an essential mineral required in humans required for building strong bones and teeth, proper blood circulation among other functions. Its deficiency leads to osteoporosis and higher risk of fracture. Therefore, this result is suggestive that leaves of *H. rosa-sinensis* can help supplement calcium requirement upon consumption. Vegetables are poor sources of iron. So, the low iron content of these processed products were not surprising as it was within the range obtained for other green leafy vegetables by Mepba et al*.* [16] and Shokunbi et al*.* [21] but differed with that of Oboh [20]. However, obvious decreases in the iron content of the extracts and the dried powdered products when compared to raw leaf indicates that the former (extracts) reduced due to effects of blanching and heating which led to the leaching off of the nutrient while the later (dried products) which had slight decrease indicates that blanching also contributed to the loss in iron content. The RDA value of Fe/day for men (19 years and older) and women (over 50 years) is 8 mg/day while that for girls and women (11 - 50

years old) is 18 mg/day [35] though neither total iron content nor nutrient density of an individual food constitutes an accurate guide for choosing dietary sources of iron but rather bioavailability and its attendant factors [16,36,37]. It seemed that blanching pre-treatment followed by extraction contributed to increased solubility and removal of soluble oxalates in the extracts, while blanching before drying caused a decrease in the oxalate content of the leaves. Blanching had been reported to induce high variations in the level of oxalates present in pretreated samples [28]. Notwithstanding, the oxalate content of the processed products was still lower than that reported for non-convectional leafy vegetables, and thus cannot be associated with renal problem or risk of renal calcium absorption [17]. Drying and blanching led to a significant reduction in the tannin level of the processed products; the trace amount of tannin found in the extract could be attributed to effects of blanching which caused the leaching off of the anti-nutrient [38] while the higher tannin content in the dried powders when compared to the extract could be attributed to dehydration leading to concentration of the tannin in the powder [25]. Tannin is known to inhibit the bioavailability of minerals and proteins by antagonistic competition and, therefore, elicit protein deficiency syndrome, kwashiorkor [26]. It hastens healing of wounds and inflamed mucous membrane due to its astringent properties or reactions [38]. Therefore, the low tannin content of these processed leaf products could be of great therapeutic advantage. Further results demonstrated that extraction is not as effective in reducing saponin content in the products as drying, hence extracted samples had higher saponin contents compared to corresponding dried samples. This may be related to the difficulties of extracting saponin from the leaf matrix. High level of dietary saponin has been found to be significant in the management of coronary heart diseases [39]. Saponin is a key component of various drug preparations and synthesis of steroid hormones [26]. It appears that blanching increased the solubility of hydrogen cyanide and enhanced its destruction. The results also indicate that extraction was more effective in reducing the cyanide contents of the products compared to drying as extracted products had significantly ($p < 0.05$) lower cyanide contents compared to dried products.

Among anti-nutrients examined in the processed products, oxalate had the highest value followed by hydrogen cyanide, tannins and saponins in that order. Yet, the oxalate and hydrogen cyanide contents were low and could possibly not cause deleterious effect on digestibility nor interfere in nutrients utilization [17]. The *H. rosa-sinensis* leaf and processed products could be considered safe with regards to acute cyanide poisoning due to the fact that the cyanide levels were far below the detrimental level of 30 mg/kg [20]. Considering the amount of anti-nutritional factors that were detected in the formulated samples, it is concluded that none of them can possibly be toxic under normal meal portions nor inhibit nutrient utilization [17,28].

5. CONCLUSION

Various heat processing techniques applied during the preparation of the processed products from *Hibiscus rosa-sinensis* leaves; caused adverse effects on the chemical composition of the processed leaf products. This was evident especially for the vitamins and minerals constitution of the processed products. More so, blanching and drying caused a significant reduction in the nutrients and anti-nutrient composition of the formulated samples. While the best processed samples were the dried powdered products, especially the RDLP, whereas the worst processed samples were the extracts, notably B_2LE. It is recommended that other processing techniques such as freezing, solar and spray drying and ethanol extraction can also be applied in order to determine their effects on nutrient retention and anti-nutrient reduction on the plant leaves and compare it with the results of this study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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